The role of histology in idiopathic pulmonary fibrosis: An update

Alberto Cavazza a,*, Giulio Rossi b, Cristiano Carbonelli c, Lucia Spaggiari d, Massimiliano Paci e, Alberto Roggeri c

a Unità Operativa di Anatomia Patologica, Ospedale Santa Maria Nuova, Viale Risorgimento 80, 42100 Reggio Emilia, Italy
b Unit of Pathology, Azienda Policlinico, Modena, Italy
c Unit of Pulmonology, Azienda Ospedaliera Santa Maria Nuova, Reggio Emilia, Italy
d Unit of Radiology, Azienda Ospedaliera Santa Maria Nuova, Reggio Emilia, Italy
e Unit of Thoracic Surgery, Azienda Ospedaliera Santa Maria Nuova, Reggio Emilia, Italy

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Review

Summary
The diagnosis of idiopathic pulmonary fibrosis (IPF) currently requires an integrated clinical—radiological—pathological approach in which the histology plays a different role from in the past. The first reason for this change is that non-invasive diagnostic procedures, particularly pulmonary function tests and high resolution computed tomography, have become increasingly competitive with biopsy in providing prognostic information. The other reason is a better appreciation of the limitations of histology: sampling error and interobserver variation. In this review we analyze the reasons for this change of perspective, provide an update on the practical role of histology in the diagnosis of IPF and discuss some of its complications.

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Introduction

In the last few years the diagnosis of idiopathic pulmonary fibrosis (IPF) has gradually shifted from a situation in which biopsy was the single gold standard to a more complex paradigm in which the histology is part of a dynamic multidisciplinary approach integrating the clinical, radiological and pathological data.1–4

The reasons for this profound change are twofold. First, the non-invasive diagnostic procedures, particularly pulmonary function tests5–9 and high resolution computed tomography (HRCT),10,11 became increasingly competitive with biopsy in providing prognostic information. Nowadays they are a sufficient surrogate of biopsy in a significant proportion of patients, and when biopsy remains necessary they integrate with histological results. For example,
when HRCT appearance is typical of usual interstitial pneumonia (UIP) a non-invasive (clinico-radiological) diagnosis of IPF is almost always accurate.\textsuperscript{12} As a corollary, biopsy is required only when the HRCT scan and/or the clinical features are not typical of UIP, a situation occurring in <50% of patients with IPF. In this scenario the correct classification of the disease is based on histology, and the histological distinction between UIP and non-specific interstitial pneumonia (NSIP) provides important prognostic information.\textsuperscript{13} In other words, when clinico-radiological data are inconclusive and biopsy is deemed necessary, histology generally remains the most important piece of the diagnostic puzzle. However, HRCT maintains a role in determining the most appropriate site of biopsy, and the prognosis is further refined when histological data are integrated with HRCT\textsuperscript{14} and clinico-functional parameters.\textsuperscript{15}

Together with the growing importance of non-invasive procedures, the other reason for the change in the role of histology in IPF is a better appreciation of its limitations. The first limit of histology is sampling errors — any disease in the lung is heterogeneous, and a biopsy in different areas may provide different results. For example, in IPF an optimal biopsy will demonstrate UIP, but a suboptimal biopsy may show only non-diagnostic honeycomb, areas of NSIP or a background of smoking-related changes. In particular, areas histologically indistinguishable from NSIP commonly occur in UIP\textsuperscript{16}: they are generally focal, but sometimes they are extensive. If the wrong area is sampled a histological diagnosis of NSIP can be misleading because the prognosis will be dictated by the non-sampled UIP.\textsuperscript{17,18}

The problems related to sampling can be reduced by obtaining an optimal biopsy (Table 1) and correlating the histology with the clinical and radiological data: if the histology does not explain the clinico-radiological scenario, the possibility that the relevant disease has not been sampled should be considered. It is important to note that the pathologist has to maintain an open mind because the histological interpretation can be modified by clinical and radiological data.

The second limit of histology in interstitial lung disease (ILD) is interobserver variation. Several recent studies evaluating this issue showed variable agreement not only among pathologists but also among clinicians and radiologists.\textsuperscript{19–23} In particular, Flaherty et al.\textsuperscript{23} reached the following conclusions: 1) interobserver agreement is better among experts than non-experts, but is not perfect even among experts; 2) agreement between experts and non-experts is variable, but in general is quite low — the field in which experts and non-experts most frequently disagree is the differential diagnosis of IPF, NSIP, collagen vascular disease (CVD) and chronic hypersensitivity pneumonitis (HP), with non-experts being more likely to assign a diagnosis of IPF; and 3) an iterative diagnostic approach improves the interobserver agreement.

It is likely that continued education and increased interaction between experts and non-experts could reduce interobserver variation\textsuperscript{22}, however both interobserver variation and sampling errors are only partially avoidable because they are intrinsic to histology. Not surprisingly, pathologists have to deal with them not only in ILD but also in many other fields.\textsuperscript{24–33} As emphasized by Wells, "... histopathologic appearances may be intermediate between two entities in a significant proportion of cases, and observer variation may be an appropriate and accurate reflection of this fact".\textsuperscript{2}

In summary, in IPF (and in ILD in general) the growing importance of non-invasive procedures and the better perception of the limits of histology (particularly sampling errors and observer variation) have gradually transformed histology from the sole gold standard to a piece of the diagnostic puzzle, a much more complex and stimulating situation for the pathologist because it requires not only correct evaluation of the histology but also its correct interpretation in light of the clinical and radiological information. In the following pages we will examine the practical role of histology in IPF, focusing on the main scenarios in which the pathologist can be involved.

### Histological diagnosis of UIP

The histological features of UIP are beautifully described in recent papers.\textsuperscript{34–36} The diagnostic keys are: 1) a patchwork appearance resulting from alternating areas of scarred and normal lung; 2) architectural distortion; and 3) fibroblastic foci.

At low magnification (Fig. 1A), the disease is non-uniform because of an irregular juxtaposition of scarred and normal or nearly normal lung (spatial heterogeneity). The scarred areas frequently prevail in the subpleural/paraseptal regions (Fig. 1B), with an abrupt transition with normal lung (patchwork pattern).\textsuperscript{16,34} The architecture is distorted, with honeycomb and thick scars obscuring the alveolar framework.

Honeycomb (Fig. 1C), which can be absent in early cases, consists of enlarged airspaces lined by bronchiolar epithelium and frequently filled by mucus and inflammatory cells, mostly neutrophils and macrophages. The background architecture is distorted, which is the key to differentiating honeycomb from the enlarged airspaces.

| Table 1 Characteristics of an optimal biopsy. |
|---------------------------------|---------------------------------|
| **Surgical biopsy**             | **Transbronchial biopsy**       |
| Accurate selection of the site of biopsy (based on computed tomography) | Accurate selection of the site of biopsy (based on computed tomography) |
| Artefacts as few as possible    | Artefacts as few as possible    |
| Multiple biopsies from at least two different areas; biopsies deep and large enough (>3 cm) | Biopsies adequate for dimension and number (at least 4–6 fragments, with alveolar parenchyma present in the majority) |
that can be seen in fibrosing NSIP (see below) and from peribronchiolar metaplasia, a frequent incidental finding in many conditions, including UIP.37 Smooth-muscle hyperplasia is frequently seen in scarred lung and can be prominent in some cases.

Fibroblastic foci (Fig. 1D) are present in the background of scarring, frequently at the interface with normal lung, and consist of small, dome-shaped interstitial collections of myofibroblasts within myxoid stroma, covered by hyperplastic pneumocytes or bronchiolar cells. Generally they are easily seen, even at low magnification, because of their pale appearance, which contrasts with the pink colour of scars. Being the site of ongoing injury fibroblastic foci indicate active disease, whereas fibrotic scars and honeycomb indicate an injury occurring in the distant past (temporal heterogeneity). In patients with IPF extensive fibroblastic foci have been associated with a particularly poor prognosis in some studies, but not all.38

Inflammation is frequently present in UIP, including small areas simulating eosinophilic pneumonia39 and occasional incidental granulomas. A cellular infiltrate including lymphoid follicles can be quite prominent in honeycomb, but outside these areas inflammation is generally minimal and overshadowed by fibrosis.

The role of special stains

Histochemical and immunohistochemical stains are invaluable in some specific settings, in particular Ziehl-Neelsen, Grocott, Gram and several immunohistochemical markers if an overinfection is a consideration; elastic stains to evaluate vessels in pulmonary hypertension; iron stains to search for asbestos bodies; and p63 or high-molecular-weight cytokeratins to differentiate adenocarcinoma from peribronchiolar metaplasia in difficult cases.40 However, none of these markers has proved useful in the diagnosis of uncomplicated UIP. Trichrome stains fibrosis, but fibrosis is generally obvious in sections routinely stained with haematoxylin—eosin.
Much more promising are immunohistochemical markers, which may be useful in difficult cases to highlight subtle modifications that may be overlooked when stained with haematoxylin–eosin. We think it is worth investigating if this level of sensitivity is practically relevant, i.e. if diagnosing IPF with immunomarkers has a superior prognostic impact to using haematoxylin–eosin. Until these studies are performed, in our opinion, the pathological diagnosis of UIP should be based on the careful evaluation of high-quality routinely stained slides. In our practice we use special stains when the artefacts are so heavy as to preclude a detailed evaluation of routine sections.

The role of transbronchial biopsy

Occasionally, transbronchial (but also transthoracic) biopsies performed in patients with IPF show histological features of UIP (Fig. 2). In the only article addressing this topic, the diagnostic sensitivity of transbronchial biopsies in UIP was about 30%. However, this was a retrospective and unblinded study in which all the patients were known to have UIP, and further prospective studies in which a variety of fibrotic ILD are blindly evaluated are clearly needed before transbronchial biopsy can be recommended in the workup of patients with suspected IPF. In our opinion, as also emphasized in the editorial accompanying the paper of Berbescu et al., until such studies are performed the histological diagnosis of UIP requires a surgical biopsy.

We also think that the claimed role of transbronchial biopsy in suggesting an alternative diagnosis is questionable in the subset of well-studied patients with idiopathic fibrosing ILD and an HRCT atypical for UIP, the setting in which a biopsy is required for the diagnosis. For example, occasional granulomas can be found in IPF as an incidental finding, and their presence is not per se diagnostic of HP or sarcoidosis if the clinico-radiological scenario does not support these possibilities. In practice, if the fibrosing ILD is considered idiopathic after an accurate clinical workup, very rarely will the results of a transbronchial biopsy be strong enough to be diagnostic and spare the patient a subsequent surgical biopsy.

Figure 2  Histological features of usual interstitial pneumonia in small biopsies. A) A generous transbronchial biopsy in an elderly male with a clinico-radiological diagnosis of idiopathic pulmonary fibrosis (haematoxylin–eosin × 20). B) A small area of patchwork pattern, with a dense scar side-by-side with normal lung (haematoxylin–eosin × 100). C) A fibroblastic focus (haematoxylin–eosin × 200). D) A transthoracic biopsy in an elderly male with a clinico-radiological diagnosis of IPF, showing a scarred lung. The biopsy was performed for a peripheral nodule, which was not sampled (haematoxylin–eosin × 20). E) Abrupt transition between scarred and normal parenchyma (haematoxylin–eosin × 20). F) A fibroblastic focus (haematoxylin–eosin × 200). Although suggestive of usual interstitial pneumonia, the diagnostic specificity of these findings is not proved.
Differential diagnosis of UIP and fibrosing NSIP

A pattern of NSIP can be found at histology in several clinical settings, particularly CVD, HP and drug reactions, or it can be idiopathic. In two recent papers\textsuperscript{13,45} 4% and 10% of patients initially considered idiopathic developed a CVD during the follow-up, and in another study\textsuperscript{46} 88% of idiopathic cases met the definition of undifferentiated connective tissue disease. In practice, a histological diagnosis of NSIP should prompt the clinician to carefully exclude a secondary form. By contrast with IPF, which typically occurs in old smokers, idiopathic NSIP prevails in middle-aged patients who have never smoked.\textsuperscript{13} Although it is not clear how much weight can be attached to this clinical difference in a single case,\textsuperscript{47,48} in practice the pathologist has to be particularly careful before making a diagnosis of UIP in a relatively young patient who never smoked or a diagnosis of NSIP in an old smoker.

### Figure 3
Histology of fibrosing non-specific interstitial pneumonia (NSIP). A) Uniform interstitial fibrosis with preservation of the alveolar architecture. At low magnification the absence of patchwork and architectural distortion are the keys to differentiating NSIP from usual interstitial pneumonia (compare with Fig. 1A). Note the absence of honeycomb and fibroblastic foci (the fibrosis is all of the same age) (haematoxylin–eosin × 20). B) Also in areas in which fibrosis is more marked, the alveolar framework is still recognizable (haematoxylin–eosin × 20). C) Sometimes in NSIP the fibrosis is looser, a feature rarely seen in usual interstitial pneumonia (haematoxylin–eosin × 40). D) Enlarged airspaces surrounded by interstitial fibrosis and lined with bronchiolar or alveolar epithelium. These enlarged airspaces are quite frequent in fibrosing NSIP and differ from honeycomb in the finer character of the fibrosis, which respects the alveolar architecture (compare with Fig. 1C) (haematoxylin–eosin × 40).

### Table 2
Contrasting histological features of usual interstitial pneumonia (UIP) and fibrosing non-specific interstitial pneumonia (NSIP).

<table>
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<tr>
<th>Character of the fibrosis</th>
<th>UIP</th>
<th>Fibrotic NSIP</th>
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<tr>
<td>Architecture</td>
<td>Non-uniform (spatial heterogeneity/patchwork)</td>
<td>Uniform</td>
</tr>
<tr>
<td>Honeycomb</td>
<td>Distorted</td>
<td>Preserved</td>
</tr>
<tr>
<td>Fibroblastic foci</td>
<td>Frequently present</td>
<td>Absent or minimal</td>
</tr>
<tr>
<td></td>
<td>Present (temporal heterogeneity)</td>
<td>Absent or very few</td>
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Microscopically, NSIP is characterized by thickening of the interstitium by inflammatory cells (lymphocytes and plasma cells) in the cellular variant, or by fibrosis in the fibrosing variant. Mixed forms occur, and are included in the fibrosing group. Whereas the distinction between UIP and cellular NSIP is straightforward, in a minority of patients the differential diagnosis between UIP and fibrosing NSIP (although clinically relevant) is difficult, with occasional cases in which a firm distinction is impossible. This generally results from poor sampling, although occasional cases are difficult to classify because they lie in the grey zone between the two entities. In these situations an open discussion between clinician, radiologist and pathologist can be particularly fruitful, but when the case remains uncertain, a second opinion may be necessary.

<table>
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<th>Table 3</th>
<th>Histological features which, when present in usual interstitial pneumonia, suggest diseases different from idiopathic pulmonary fibrosis (IPF).</th>
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<tr>
<td>Histological feature</td>
<td>Consider</td>
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<tr>
<td>Cellular lymphoplasmacytic infiltrate and/or cellular bronchiolitis and/or lymphoid follicles</td>
<td>Collagen vascular disease, chronic hypersensitivity pneumonitis</td>
</tr>
<tr>
<td>Peribronchiolar fibrosis (sometimes bridging to the periphery of the lobule)</td>
<td>Chronic hypersensitivity pneumonitis, pneumoconiosis</td>
</tr>
<tr>
<td>Pleuritis</td>
<td>Collagen vascular disease</td>
</tr>
<tr>
<td>Granulomas</td>
<td>Depending on the morphology and localization of granulomas: infection (particularly atypical mycobacterial infection secondary to traction bronchiectasis), chronic hypersensitivity pneumonitis, chronic sarcoidosis, IPF arising in a background of incidental sarcoidosis, incidental finding in IPF</td>
</tr>
<tr>
<td></td>
<td>Asbestosis (do iron stains on several slides to search for asbestos bodies, which can be few), chronic haemorrhage (consider the possibility of ANCA-associated fibrosis)</td>
</tr>
<tr>
<td>Abundant coarse iron pigment</td>
<td>Drug reaction (focal areas resembling eosinophilic pneumonia are an unusual incidental finding in IPF)</td>
</tr>
<tr>
<td>Foamy macrophages with eosinophils</td>
<td></td>
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Figure 4  Examples of secondary usual interstitial pneumonia. A) Surgical lung biopsy in a middle-aged woman with rheumatoid arthritis (case courtesy of Prof. T.V. Colby, Scottsdale, USA). Note the numerous lymphoid follicles with germinal centres, which are the clue to suspecting an underlying collagen vascular disease (haematoxylin–eosin × 20). B) Surgical lung biopsy in an elderly farmer. The centrolobular involvement with fibrosis bridging to the periphery of the lobule, and the small peribronchiolar granuloma (insert), are characteristic of chronic hypersensitivity pneumonitis (haematoxylin–eosin × 20).
unsolved it may be advisable to refer the patient (or the slides) to a centre with expertise in ILD. The histology of fibrosing NSIP is shown in Fig. 3, and the contrasting features with UIP are presented in Table 2.

Differential diagnosis of IPF and secondary UIP

In addition to IPF other diseases that may present with a UIP pattern include CVD, chronic HP, asbestosis, drug reaction and familial ILD. In some cases these diseases are perfect histological mimics of IPF and the distinction is based exclusively on clinical grounds. In other cases there are histological features (sometimes subtle) that may suggest the possibility of one of these diseases (Table 3, Fig. 4). Importantly, none of these features is diagnostic per se and the final diagnosis should always rest on the careful correlation of the histology with the clinical data. Nonetheless, it is important for the pathologist to suggest (when it is possible) a secondary UIP rather than IPF because the distinction may have prognostic implications in some settings, although it is probably less

![Figure 5](image_url)

**Figure 5** Histology of smoking-related fibrosis and chronic Langerhans' cell histiocytosis (LCH). A) Dense ropy collagen typical of smoking-related fibrosis (haematoxylin–eosin × 40). B) Smoking-related fibrosis frequently surrounds enlarged airspaces, both in subpleural and centrolobular regions. In the centre-right of the picture note the intra-alveolar accumulation of pigmented macrophages (respiratory bronchiolitis) (haematoxylin–eosin × 20). C) Stellate peribronchiolar scar typical of chronic LCH, causing traction emphysema. This kind of fibrosis, totally different from usual interstitial pneumonia, is diagnostic of chronic LCH even in the absence of residual Langerhans’ cells (haematoxylin–eosin × 20). D) Pigmented macrophages entrapped within fibrosis. Although not entirely diagnostic, this feature is characteristic of LCH (haematoxylin–eosin × 200).

![Figure 6](image_url)

**Figure 6** Surgical lung biopsy showing usual interstitial pneumonia with numerous fibroblastic foci and organizing pneumonia. A case like this lies somewhere on the spectrum between stable idiopathic pulmonary fibrosis and acute exacerbation. This biopsy was performed for a recent worsening of symptoms, but the patient did not satisfy the clinical criteria for acute exacerbation (haematoxylin–eosin × 40).
meaningful in others. The field is still controversial, but the presence of UIP at histology seems to predict a poor survival, similar to IPF in patients with rheumatoid arthritis and chronic HP, whereas the prognosis seems better than IPF when UIP is related to other CVD.\textsuperscript{51,67}

**Diagnosis of UIP against a background of smoking-related changes**

Alongside the more frequent chronic bronchitis, emphysema, bronchogenic carcinoma and accumulation of finely pigmented macrophages (respiratory bronchiolitis),\textsuperscript{58} cigarette smoking can cause fibrosis, sometimes significant, which is part of the morphological spectrum of smoking-related ILD (Fig. 5A and B).\textsuperscript{69–72} In some cases the diagnosis of early UIP arising on a background of smoking-related fibrosis can be challenging. To complicate matters, in a recent paper in which the background parenchyma of specimens resected for lung cancer was carefully evaluated, foci of UIP were found in 3.5% of non-smokers, 15.4% of mild smokers, 23.6% of moderate smokers and 22.4% of heavy smokers.\textsuperscript{72} The patients for the most part had no clinical evidence of ILD, and whether these foci of UIP corresponded to early/subclinical IPF or were just incidental findings is not known. Interestingly, acute respiratory failure following surgery developed only in patients with foci of UIP.\textsuperscript{72}
Acute exacerbation of IPF and other fibrosing ILD

Some patients with IPF (but also with other fibrosing ILD, including idiopathic NSIP,75–77 chronic HP75,76,79 and ILD related to CVD76,77,80,81) experience episodes of acute deterioration of their illness with a high mortality rate. When idiopathic these episodes are called acute exacerbation (AE).82–87 The frequency of AE in IPF is not known. A recent retrospective review on 147 patients with IPF84 showed a 2-year incidence of AE of 9.6%; surprisingly, patients with less extensive opacities at HRCT91 and with OP rather than DAD at histology75,76,90 represent the most severe end of a spectrum. Not surprisingly, patients with less extensive opacities at HRCT91 and with OP rather than DAD at histology75,76,90 have less severe disease and a better outcome.

Carcinoma in IPF

Patients with IPF or other fibrosing ILD are at increased risk of developing pulmonary carcinoma. Preliminary data suggest that squamous cell carcinoma92 and unusual variants of adenocarcinoma, including the enteric type93 (Chiosis M, personal communication) are more frequently seen in IPF than the general population. The diagnosis of malignancy in this setting is generally straightforward but occasionally very difficult, particularly on small biopsies, but sometimes also on surgical specimens.94–95 Some pulmonary adenocarcinomas are so well differentiated they are difficult to appreciate as neoplastic, in particular if the tumour cells are few and masked by fibrosis; moreover, some reactive conditions, particularly pneumocyte hyperplasia and peribronchiolar metaplasia, can be so exuberant as to closely mimic a tumour. Some examples are shown in Fig. 7, and the criteria for differentiating adenocarcinoma from reactive conditions in ILD are summarized in Table 4.

Conflict of interest statement

The authors have no conflicts of interest to declare.
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